

Supplementary Information

Sustainable development of pellucid bio-slide from discarded fish scales for microscopy imaging

Amal M E^{±,a}, K. P. Paul David^{±,a}, Aisharwaya D. Bantupalli^{#,a}, Elna B. Kuriappuram^{#,a}, Ambika Ray^{#,a}, Venkata Sruthi Kadiyala^a, Jemi Feiona Vergil Andrews^a, Salman Khan^a, Ciranjeevi Korupalli^a, Vengala Rao Yenuganti^a, Madhubabu GB^b, Pitchaiah Cherukuri^a, Divya S. Parimi^a and Anil K. Suresh^{a,*}

^aDepartment of Biological Sciences, School of Engineering and Applied Sciences, SRM University-AP, Amaravati-522502, India.

^bDepartment of Biotechnology and Bioinformatics, University of Hyderabad, Hyderabad-500046, India.

**Corresponding author:*

E-mail: anil.s@srmmap.edu.in (Anil K. Suresh)

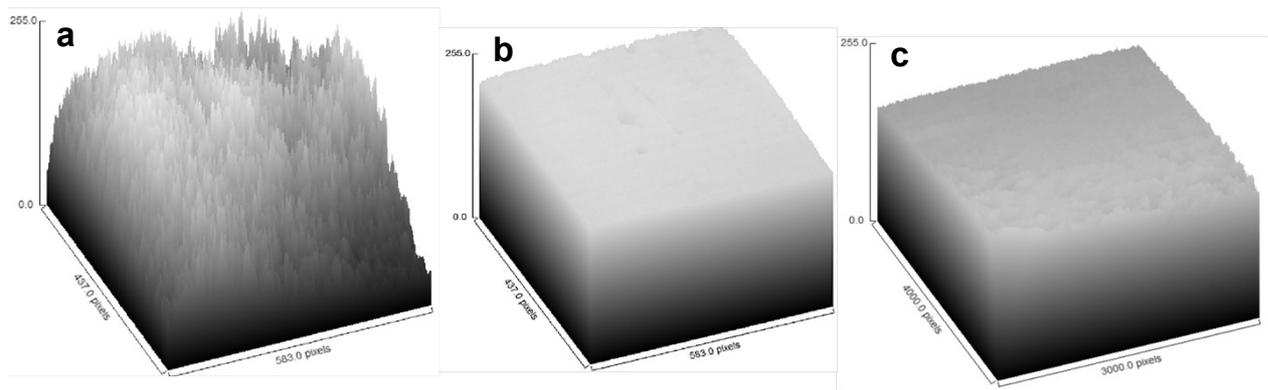


Fig. S1. Surface roughness analysis of our bio-slide coated in comparison with glass slide. Surface roughness analysis of (a) pristine unprocessed fish scales, (b) bio-slide and (c) glass slide as obtained from the microscopy images using ImageJ. The plot was obtained based on pixel analysis (1 pixel = 0.0005 mm to 0.0014 mm) for a particular region chosen from the microscopic images. Comparative smoothness of the bio-slide (b) to that of glass slide (c) can be clearly observed.

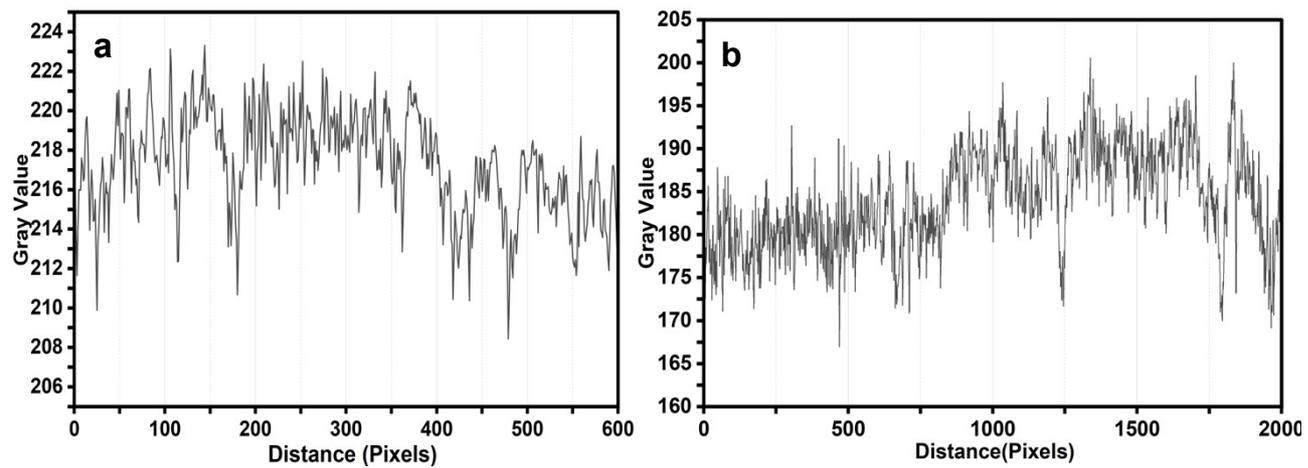


Fig. S2. Depth analysis of our bio-slide (a) and glass slide (b). No much difference in the depths was observed corroborating with the surface roughness analysis (Figure 1).

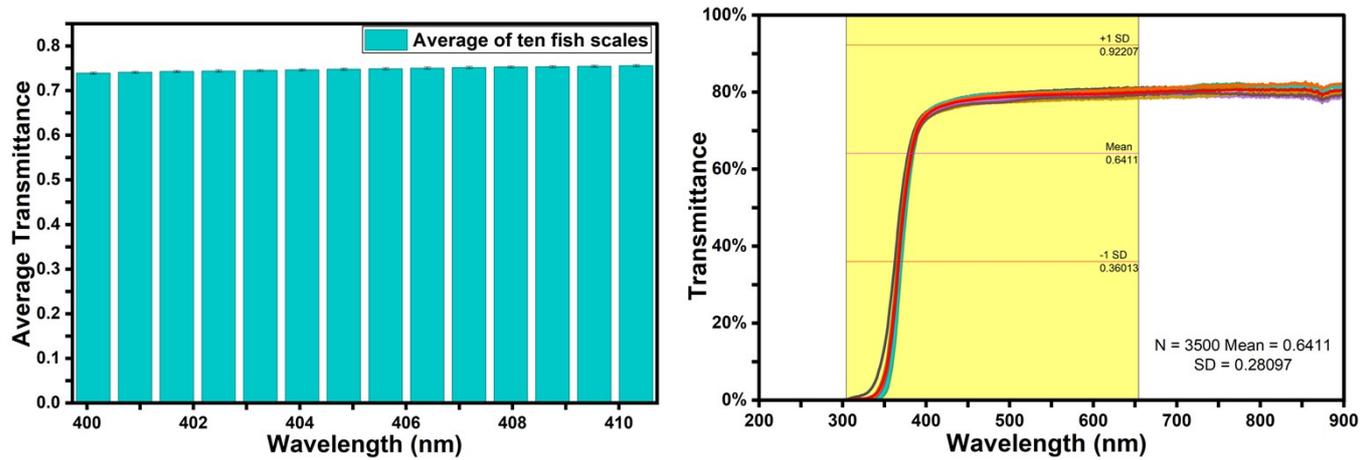


Fig. S3. Statistical analysis of transmittance of bio-slide ($n=10$).

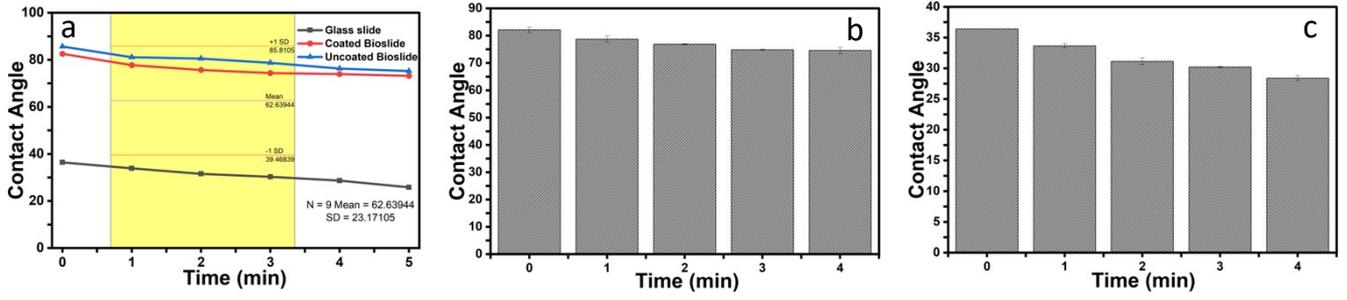


Fig. S4. Statistical analysis of contact angle on bio-slide ($n=9$). (a) Contact angle for coated and uncoated bio-slide, glass slide with mean and standard deviation. (b) Contact angle of bio-slide (b) and glass slide (c) with error bars ($n=3$).

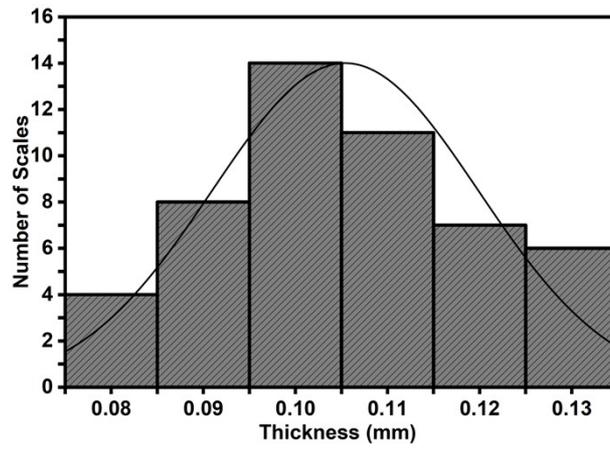


Fig. S5. Thickness calculated from 50 bio-slides as measured using a screw gauge instrument. The bio-slides were observed to be of an average thickness of 0.10 ± 0.02 .

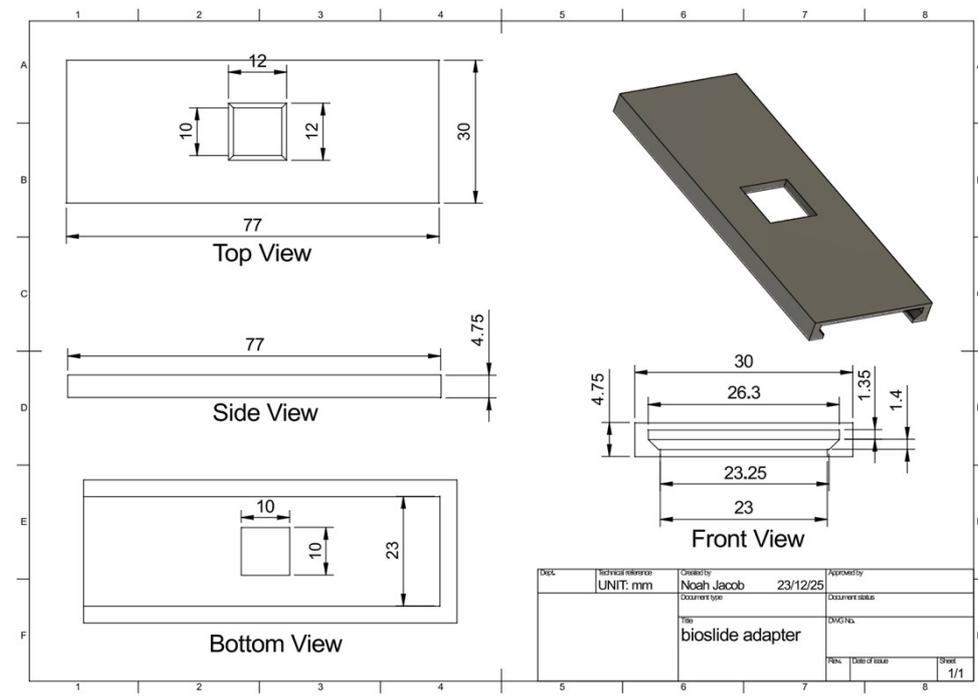


Fig. S6. CAD model dimensions and information on the materials used to print the adapters using 3D printing.

Sl. No	Material Property	Value
1.	Name	Polylactic acid (PLA)
2.	Thermal property	Thermoplastic polyester
3.	Density	1.24 g/cm ³
4.	Glass transition (Tg)	55-60 °C
5.	Melting point	200 °C
6.	Mechanical Strength	60 mPa
7.	Sustainability	Biodegradable and made from renewable plant derived sugars/starch

Table showing the various properties of the materials used for the 3D printing of the adapter.

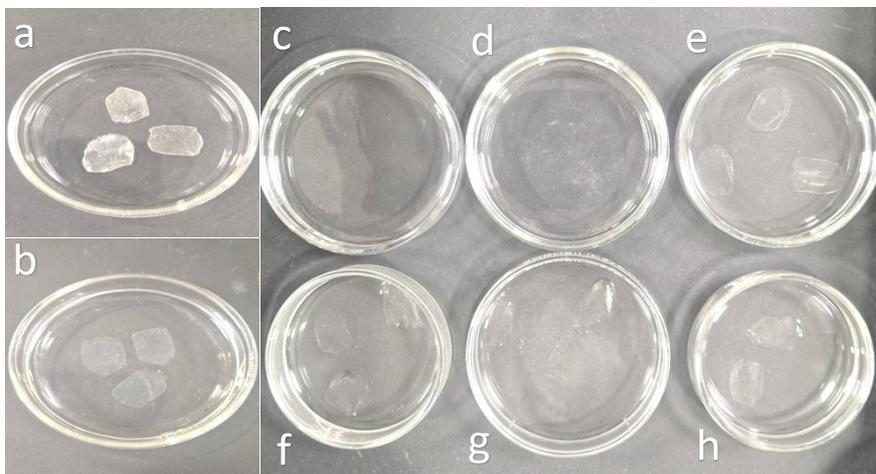


Fig. S7. Solvent stability and compatibility of our bio-slide. Photographs of the bio-slide before (a) and after immersion in different polar and non-polar solvents for 12 hours. Solvent used are (b) water (c) hexane (d) chloroform (e) acetone (f) isopropanol (g) toluene (h) chlorobenzene. The bio-slide was highly stable in all the solvents without corrosion or deuteration.

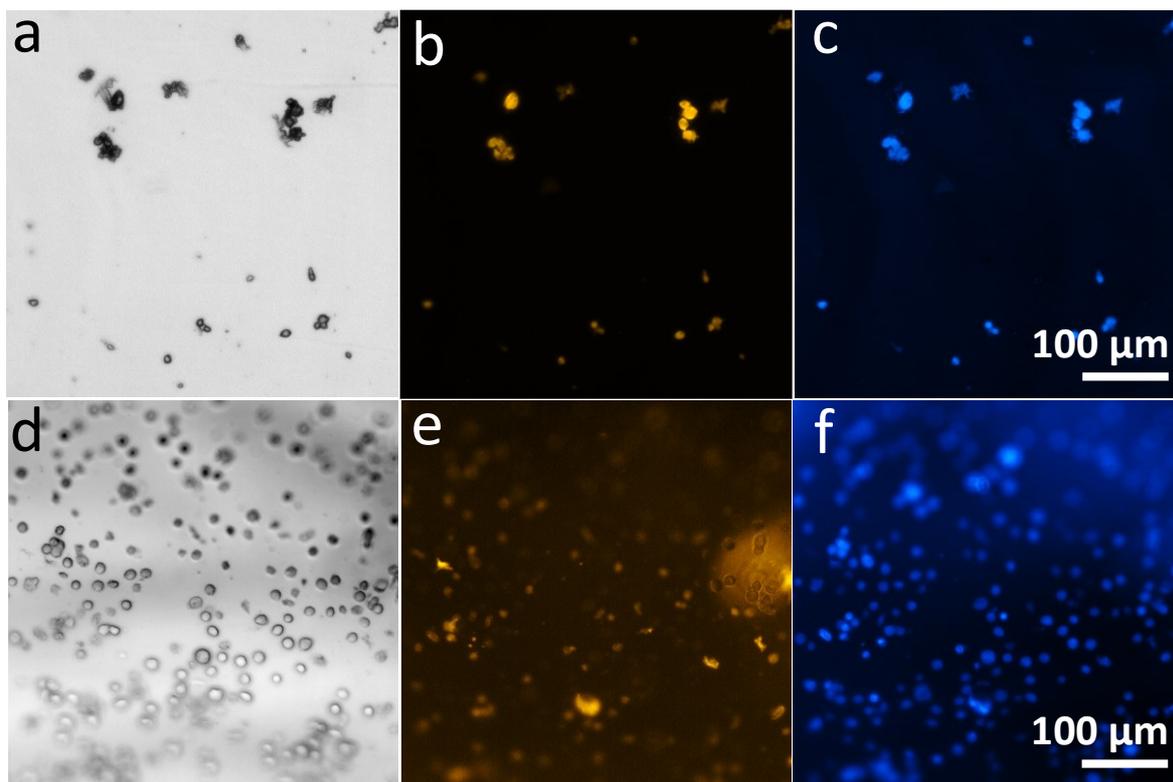


Fig. S8. Microscopy images of HeLa on bio-slides cell under bright field (a) TRITC (b) and c. DAPI (c) to their counter comparison taken on glass slides (d-f).

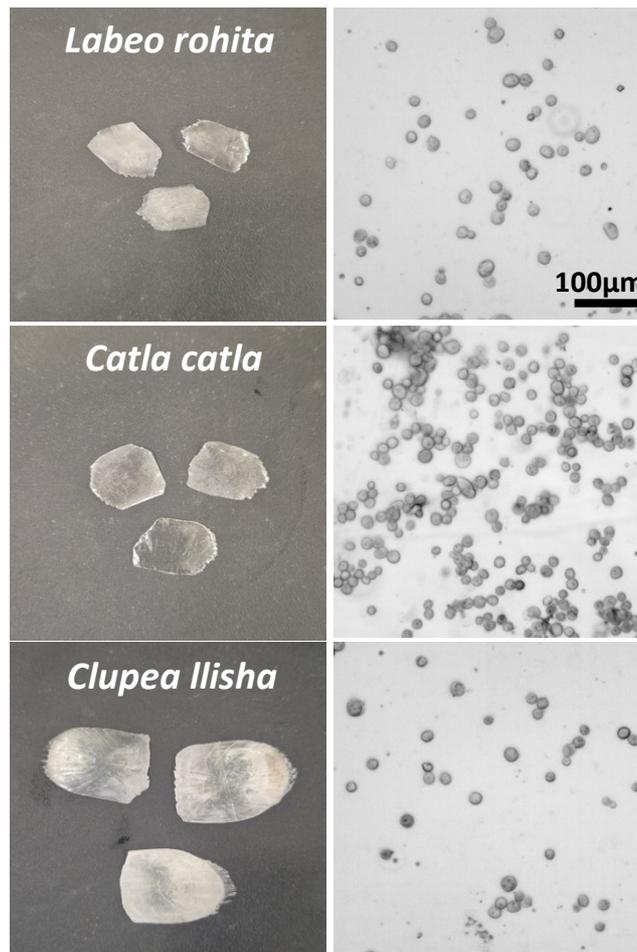


Fig. S9. Experimental demonstration of the consistent production of transparent bio-slides using scales extracted from various species; Labeo rohita and Catla catla and Clupea ilisha and their use in microscopy imaging.

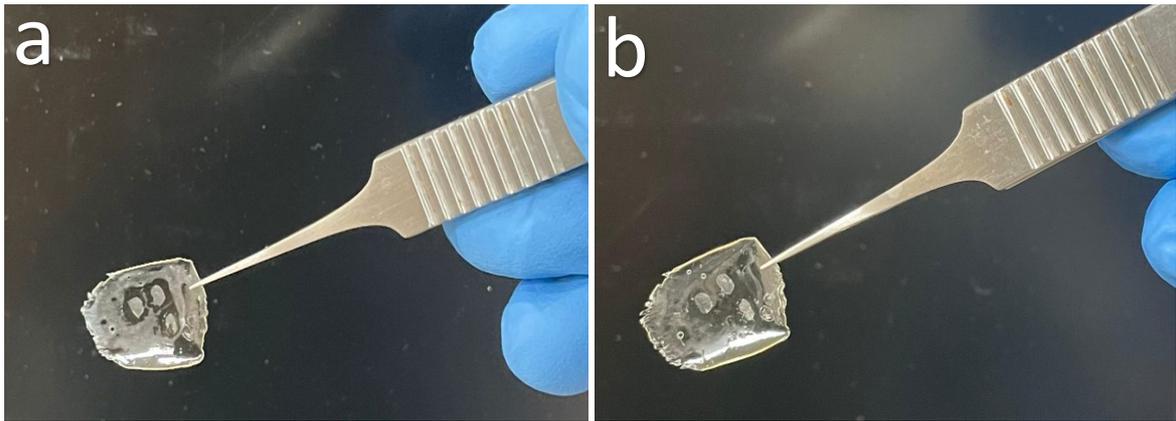


Fig. S10. Retainment of spinal cord specimen (n=3) on the bio-slide after rigorous washing and procedural steps to shown their tough adhesion onto the bio-slide.

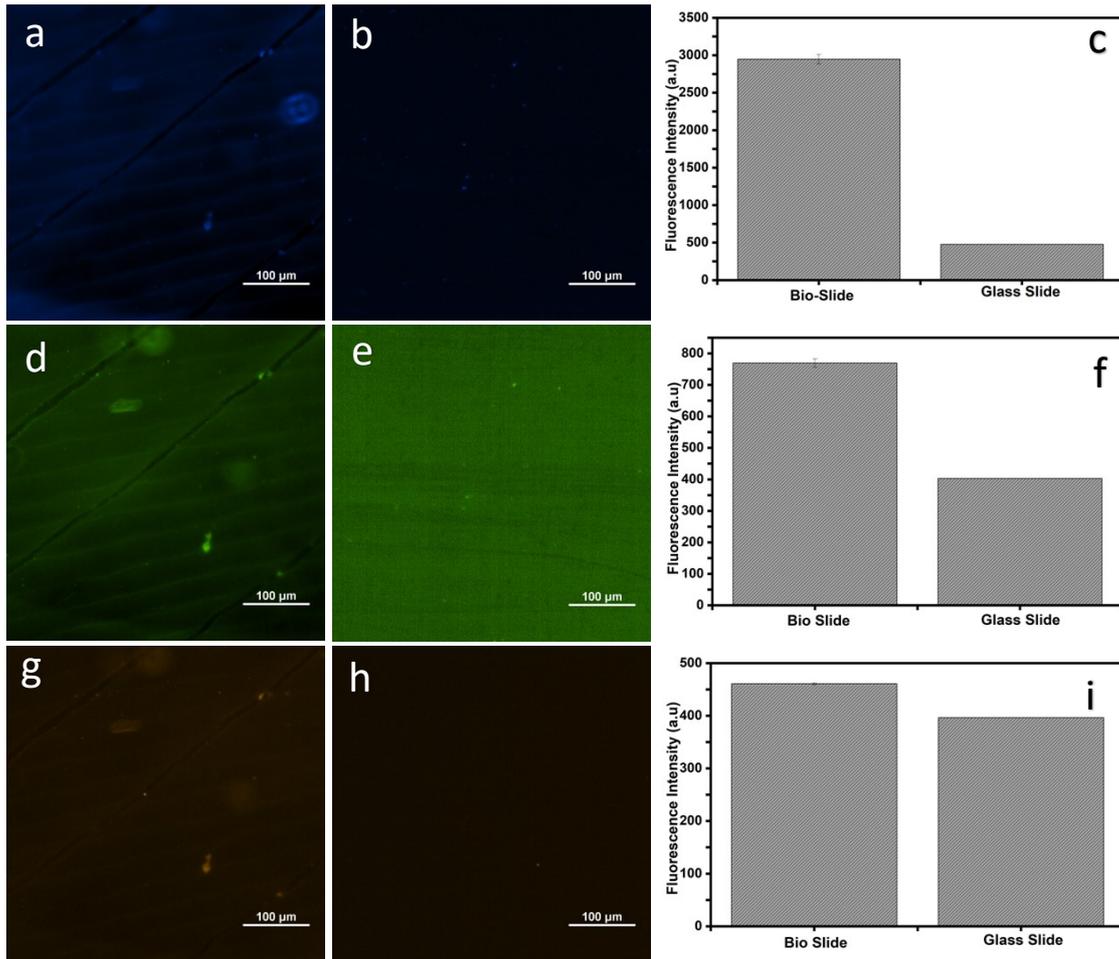


Fig. S11. Autofluorescence intensity measurements on bio-slides and glass slides. (a, d, g) represent the images from bio-slide and (b, e, h) represent images from the glass slide. (a, b) represent images in UV channel and the mean fluorescence intensities ($n=10$ ROIs) (c, d, e) represent images in FITC channel and the mean fluorescence intensities ($n=10$ ROIs) (f, g, h) represent images in TRITC channel and the mean fluorescence intensities ($n=10$ ROIs) (i).

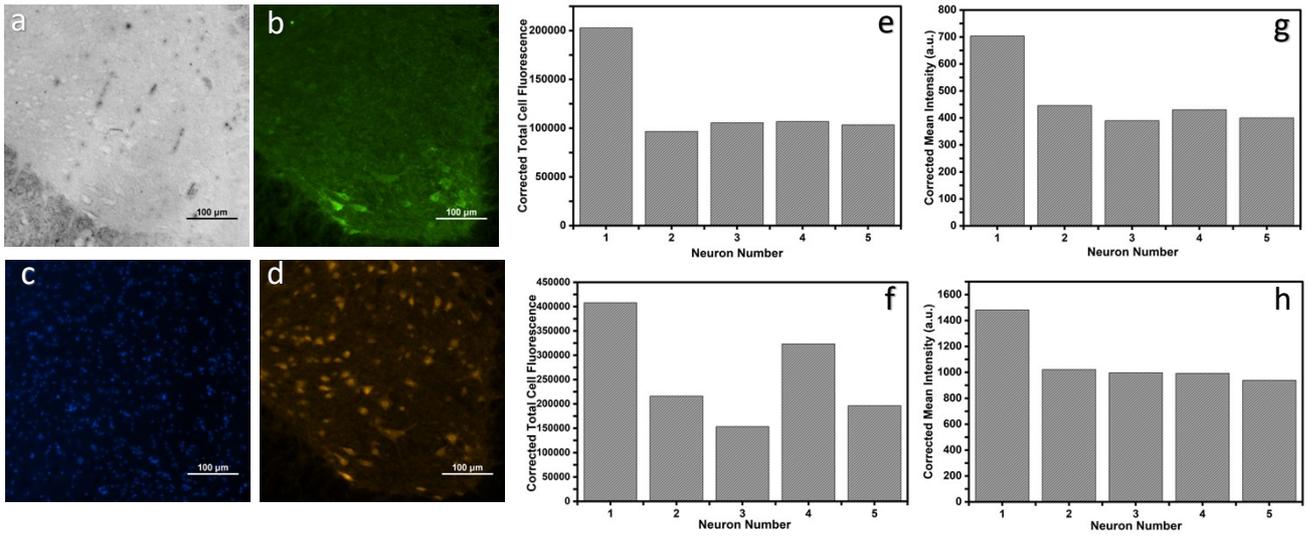


Figure S12. Fluorescence intensity measurements of 30 μm thick spinal cord sections on bio-slides. (a) bright field (b) FITC channel (c) DAPI channel (d) TRITC channel. Graphs represent total corrected neuronal cell fluorescence (n=5) from FITC (e) and TRITC (f) channels. (g, h) represent mean intensity (n=5) after background subtraction.

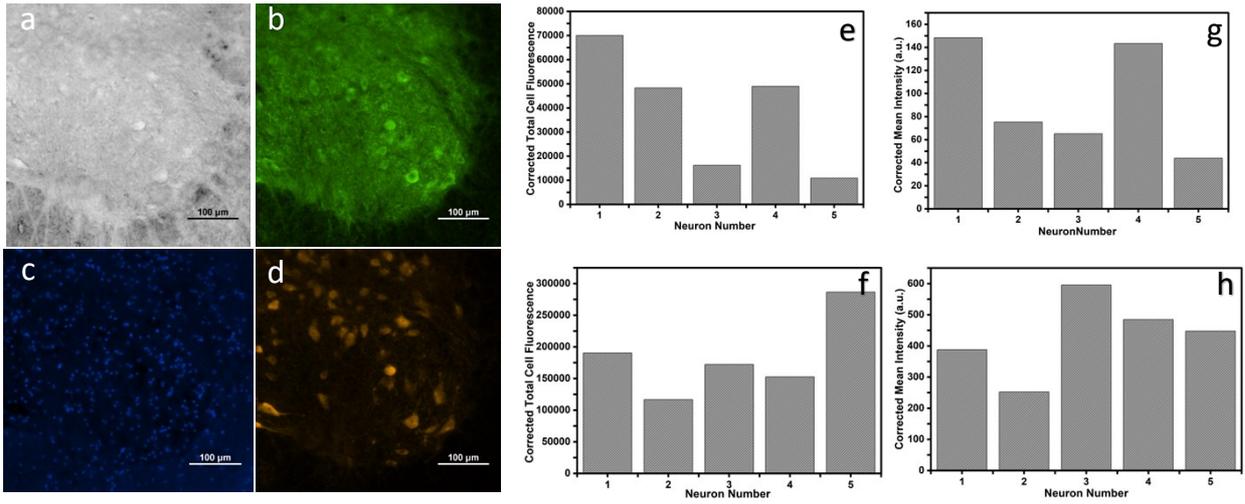


Figure S13. Fluorescence intensity measurements of 30 μm thick spinal cord sections on glass slides. (a) bright field (b) FITC channel (c) DAPI channel (d) TRITC channel. Graphs represent total corrected neuronal cell fluorescence (n=5) from FITC (e) and TRITC (f) channels. (g, h) represent mean intensity (n=5) after background subtraction.